Automated Charge State Determination of Complex Isotope-Resolved Mass Spectra by Peak-Target Fourier Transform

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This study describes a new algorithm for charge state determination of complex isotope-resolved mass spectra. This algorithm is based on peak-target Fourier transform (PTFT) of isotope packets. It is modified from the widely used Fourier transform method because Fourier transform may give ambiguous charge state assignment for low signal-to-noise ratio (S/N) or overlapping isotopic clusters. The PTFT algorithm applies a novel "folding" strategy to enhance peaks that are symmetrically spaced about the targeted peak before applying the FT. The "folding" strategy multiplies each point to the high-*m/z* side of the targeted peak by its counterpart on the low-*m/z* side. A Fourier transform of this "folded" spectrum is thus simplified, emphasizing the charge state of the "chosen" ion, whereas ions of other charge states contribute less to the transformed data. An intensity-dependent technique is also proposed for charge state determination from frequency signals. The performance of PTFT is demonstrated using experimental electrospray ionization Fourier transform ion cyclotron resonance mass spectra. The results show that PTFT is robust for charge state determination of low S/N and overlapping isotopic clusters, and also useful for manual verification of potential hidden isotopic clusters that may be missed by the current analysis algorithms, i.e., AID-MS or THRASH. (J Am Soc Mass Spectrom 2008, 19, 46–54) © 2008 American Society for Mass Spectrometry

Electrospray ionization mass spectrometry (ESI-MS) [1–4] greatly extends the capability for measuring the masses of large biomolecules by generating multiply charged ions. Multiple charging shifts the signal of molecules as large as 200 kDa down to the mass-to-charge ratio (*m/z*) for facilitated detection by most mass analyzers [5]. However, it also complicates mass spectral interpretation because the charge state of each ion must be assigned to enable determination of ion mass, and ions of a given mass typically exhibit several charge states.

Charge state determination of a fully resolved isotopic cluster is relatively straightforward because the spacing between adjacent isotopic peaks is simply the reciprocal of the charge state [6]. However, mass spectra of large biomolecules are usually very complicated because thousands of isotopic peaks from a mixture of ions are packed in an *m/z* range from 500 to 18,000 Da [7]. Consequently, this produces a high probability that two or more isotopic clusters may overlap together. The determination of charge states of overlapping isotopic clusters is one of the greatest challenges for speeding up the interpretation of complex high-resolution mass spectra.

Some efforts have been made to infer charge state determination from high-resolution mass spectra. Senko and colleagues [5, 8] described a method that combines a Patterson pattern-recognition algorithm with a Fourier transform for charge determination of fragment ions from isotopically resolved collision-induced dissociation tandem mass spectra of intact proteins in a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer [9, 10]. Their results suggest that the Patterson algorithm performs best on low charge ($Z < 5$) with low signal-to-noise ratio (S/N) and excessive resolving power. In contrast, the Fourier algorithm becomes superior on higher charge states ($Z > 5$) with low resolving power. They also demonstrated a successful example for charge determination of two overlapping isotopic clusters with significant abundance differences using Fourier transform, although the Patterson routine is not recommended because the broad peaks it produces will blend together and potentially produce inaccurate results. Zhang and Marshall [11] described a Z-score algorithm for routine charge state determination and spectral deconvolution, for either high- or low-resolution mass-to-charge ratio spectra, to yield unambiguous zero-charge mass spectra. Z-score assigns a score for each possible charge state according to an appropriate charge-scoring scheme, and then determines the charge state with the highest score as the correct one. Tabb and colleagues [6] investigated the Fourier transform approach for determining charge states from either mass spectra of peptides from a linear quadrupole ion trap mass spectrometer or mass spectra of intact proteins from an FTICR mass spectrometer. To

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our dismay, study on the overlapping isotopic clusters was not performed. Horn and co-workers [12] developed a THRASH (thorough high-resolution analysis of spectra by Horn) algorithm for automated reduction and interpretation of high-resolution electrospray mass spectra of large molecules. This method uses the Fourier transform/Patterson method for primary charge determination in each moving window. However, if the ambiguous charge states are obtained by Fourier transform/Patterson, it is required to search each possible charge state in a user-defined charge range (i.e., 1–30). The charge state with the highest reliability value is assigned as the correct charge state. In our previous research, an AID-MS (*a*utomated *i*ntensity *d*escent algorithm for analysis of *m*ass *s*pectrometry) algorithm [7, 14] for speeding up the interpretation of complex high-resolution mass spectra was developed. The results showed that AID-MS is fast in computational speed, robust in identification of overlapping isotopic clusters, and efficient in minimization of false positives. A peak selection method is proposed to identify isotopic peaks, and then the exact value or a searching range of charge state is determined by the reciprocal of the interpeak spacing of isotopic peaks. If a searching range is obtained, the charge state is determined by the optimal matching between *averagine* peaks with isotopic peaks.

Here, we present a simple and fast algorithm based on peak-target Fourier transform (PTFT) for automated charge state determination of isotope-resolved mass spectra. The PTFT algorithm selects a targeted peak in a studied *m/z* window, and then applies a "folding" strategy on the low-*m/z* side and high-*m/z* side of the targeted peak to generate a "folded" spectrum. The charge state is determined from the Fourier transform of this "folded" spectrum. The performance of the PTFT algorithm is demonstrated using complex isotopically resolved mass spectra. The results show that PTFT can identify the charge states with high accuracy, especially for low S/N and overlapping isotopic clusters.

Experimental

All examples of isotopic clusters are selected from three high-resolution FTMS spectra of different complexities, which had been analyzed by AID-MS in our previous study [7, 14]. Spectrum A is an FTICR mass spectrum of a liquid chromatography–separated fraction of commercial acid-extracted histone mixture (Sigma Chemicals) recorded by Bruker Daltonics 9.4T Q-FTMS. The other two spectra were selected from previously published plasma electron capture dissociation (ECD) of large proteins recorded by Cornell 6T FTMS [13]. Spectrum B is a plasma ECD spectrum of ubiquitin and Spectrum C is a plasma ECD spectrum of carbonic anhydrase. All of the time-domain spectra were processed by two zero-fill and no apodization. More details have been described in our previous study. The MATLAB source codes of the PTFT algorithm can be requested from the corresponding author.

Algorithm

For a theoretical isotopic cluster, the spacing between adjacent isotopic peaks is equidistant, and the charge state can be simply determined by the reciprocal of the spacing. However, due to the interference of noise signals and overlapping complexities, experimental isotopic peaks may not exhibit exactly equal spacing. Normally, a tolerance of peak shifting is required when selecting isotopic peaks from spectra; thus, the interpeak spacing between different adjacent isotopic peaks may not be same. When using the reciprocal of the interpeak spacing to estimate the charge state, the errors may be produced. When charge state (Z) is high, the trivial errors of spacing may cause a huge difference in charge states because $1/Z$ and $1/(Z + 1)$ differ only slightly. Therefore, higher charge states may give rise to higher error rates. However, it is worth pointing out that the reciprocal of the interpeak spacing works very well for the ions with low charge states.

The Fourier transform (FT) provides a means for determining the frequency of the periodic features. As described earlier, isotopic peaks of an ion are spaced by $1/Z$ in the isotope-resolved mass spectrum, so Fourier transform of isotope packets can generate useful frequency signals related to the charge state. As shown in Figure 1a and b, FT is applied to an isolated isotopic cluster with the charge state 5^+. It is observed that its frequency signal exhibits a series of peaks spaced by the charge state. The "DC" signal at zero frequency is ignored because $Z < 1$ is not allowable. Generally, the first peak in the frequency signal corresponds to the charge state, and the second peak gives the double of the charge state, and so on. However, with the addition of noise signals in-between the isotopic peaks, the frequency peaks may be distorted and not exhibit a clear pattern as shown in Figure 1c and d. Inaccurate assignment of charge states may be caused. When two or more isotopic clusters are overlapped, FT may fail to determine the charge state using the frequency signal. An example of three overlapping isotopic clusters is shown in Figure 1e and f. The FT of overlapping clusters illustrates a messy frequency signal without an obvious pattern, from which it is very difficult to determine the charge state.

We develop a novel algorithm based on peak-target Fourier transform for fast charge state determination of complex overlapping isotopic clusters. To illustrate the use of PTFT for charge state determination of ions, two overlapping isotopic clusters with known charge states (5^+ and 8^+) are simulated and the frequency signal is achieved by FT as shown in Figure 2a and b. It is known that the intensities of frequency signals are proportional to the abundances of isotopic clusters. When the abundances of two overlapping isotopic clusters differ substantially, the FT still can indicate an accurate charge state for the most abundant cluster as investigated by Senko and colleagues [5]. However, if the abundances of overlapping isotopic clusters are comparable or more than

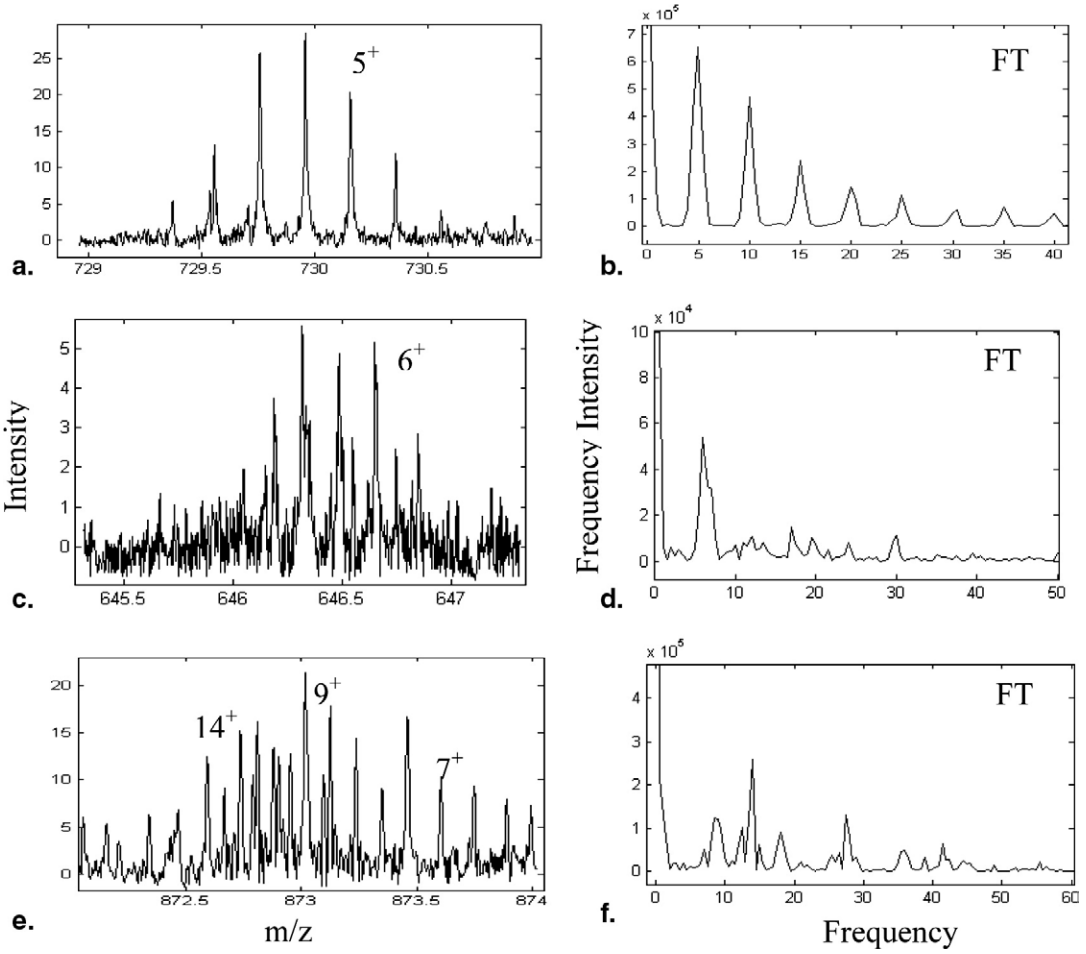

Figure 1. Charge state determination using Fourier transform (FT). (1) An isolated isotopic cluster (**a**) and its frequency signal (**b**). (2) A noisy isotopic cluster (**c**) and its frequency signal (**d**). (3) A spectrum with three overlapping isotopic clusters (**e**) and its frequency signal (**f**).

two isotopic clusters overlap together, the FT will give ambiguous results for charge state determination and the corresponding isotopic cluster of the first frequency peak or the maximum frequency peak is also not clear.

Consequently, the PTFT algorithm is proposed for charge state determination especial for low S/N and overlapping isotopic clusters. First, the algorithm selects a targeted peak in the studied *m/z* window. The spectrum is separated into two parts: the low-*m/z* side spectrum and the high-*m/z* side spectrum of the targeted peak. The low-*m/z* side spectrum is folded on the high-*m/z* side spectrum according to the targeted peak. As a result, a new spectrum is generated by array multiplication of both spectra. This operation can be illustrated as follows:

$$P = L.*R \tag{1}$$

where .* denotes the element-by-element multiplication of two vectors. L is the low-*m/z* side spectrum $[l_1, l_2, \ldots, l_n]$ and R is the high-*m/z* side spectrum $[r_1, r_2, \ldots, r_m]$. l_1 and r_1 are the position of the target peak. If the lengths of L and R are not same, the first $mn = \min\{n, m\}$ elements of the longer spectrum are used for array multiplication in eq 1. $P = [l_1 \times r_1, l_2 \times r_2, \ldots, l_{mn} \times r_{mn}]$ is the "folded" spectrum after peak targeting.

Figure 2c is the "folded" spectrum by targeting Peak 1. We can observe that only the symmetric peaks of both low-*m/z* and high-*m/z* sides are kept, whereas all asymmetric peaks are cancelled out by array multiplication. This operation will remove most of peaks that are not related to the cluster of the target peak; thus, the frequency signal of the "folded" spectrum is filtered to a great extent. In this example, because the targeted peak (Peak 1) is shared by two overlapping isotopic clusters, a small residue peak at 2000.596 *m/z* due to another cluster is still observed in the "folded" spectrum after array multiplication. However, the Fourier transform of the "folded" spectrum still shows a perfect frequency signal (Figure 2d), in which all the peaks are spaced by the charge state 8^+. Using the charge state and the targeted peak, other isotopic peaks of this cluster can be simply selected by 1/Z as shown in Figure 2a. Similarly, by targeting Peak 2, another cluster with the charge state 5^+ is successfully determined as illustrated in Figure 2e and f.

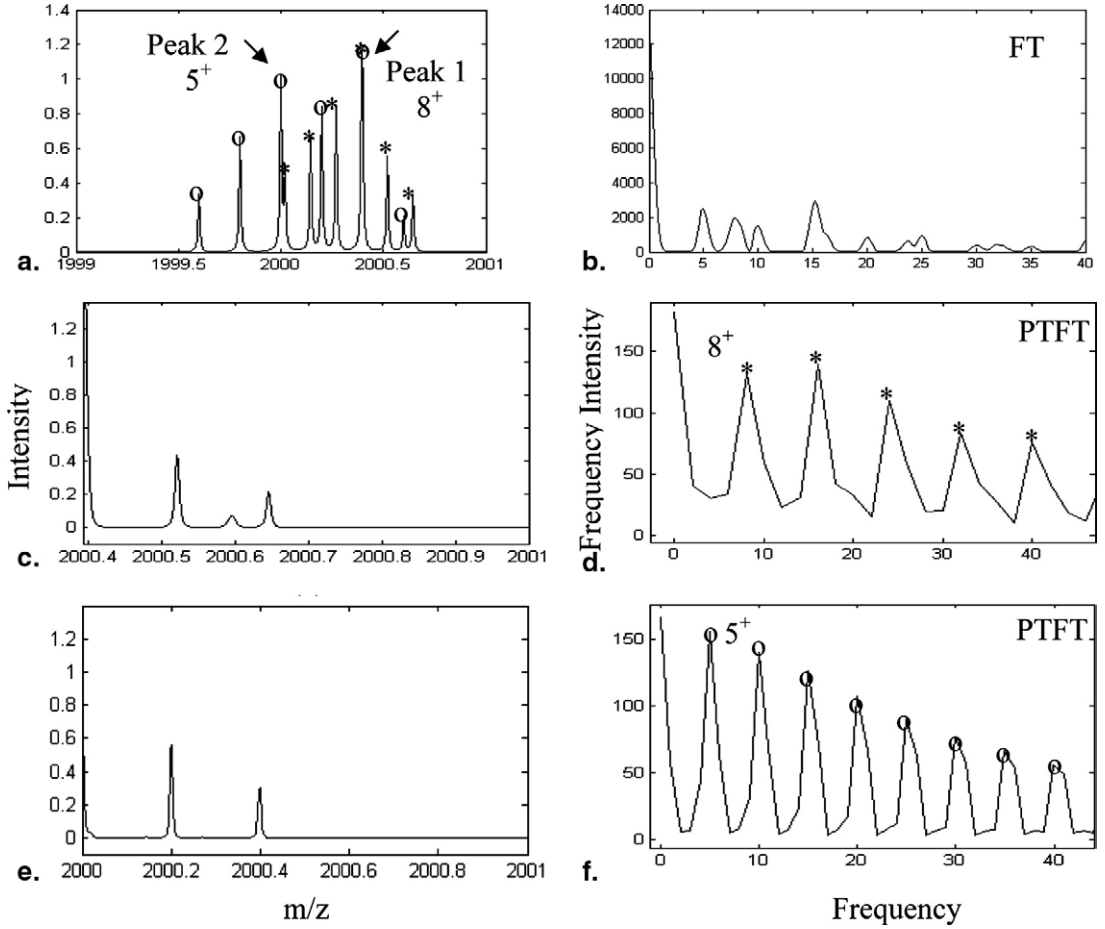

Figure 2. Peak-target Fourier transform of two overlapping simulated isotopic clusters. (1) Two overlapping isotopic clusters (**a**) and its frequency signal of FT (**b**). (2) The "folded" spectrum after targeting Peak 1 (**c**) and its frequency signal (**d**). (3) The "folded" spectrum after targeting Peak 2 (**e**) and its frequency signal (**f**).

The selection of targeted peaks is simple. For an automated analysis, the targeted peaks are selected according to their descending peak intensities. First, all of the peak maxima in the studied *m/z* range are picked and saved in a peak list. The maximum peak in this list is selected as the targeted peak. PTFT is applied to the spectrum. If an isotopic cluster is found by PTFT, the selected isotopic peaks of this cluster are removed from the peak list. If no isotopic cluster is identified, however, only the targeted peak is removed from the peak list. The above procedure is repeated until no more maximum peak in the peak list is above the intensity threshold (i.e., 10% of the maximum intensity). The charge states of all isotopic clusters are subsequently identified.

For manual verification of potential hidden isotopic clusters, the targeted peaks are user-defined. In general, we can select the center peak in a suspicious isotopic cluster as the targeted peak to make sure more peaks can be obtained in the "folded" spectrum after array multiplication. However, for the overlapping regions, we have found that use of the split peaks as targeted peaks is useful to reveal potential hidden isotopic clusters buried under the neighboring strong isotopic clusters.

In addition, we also develop a new technique to determine charge states from the frequency signals. As mentioned earlier, the first peak in the frequency signal corresponds to the charge state, and the second peak gives the double of the charge state, and so on. Because of the interference of noise signals or other overlapping isotopic peaks, the first frequency peak may be distorted. The charge state indicated by the first peak maximum may not be a correct assignment. Therefore, in our technique, the charge states contributing to the shape of the first peak are considered as possible charge states. If the number of possible charge states is more than five, only the top five charge states are used for further calculation. The intensities of frequency peaks above a user-defined threshold (i.e., 10% of the intensity of the first peak) with the interval of each possible charge state are summed. The charge state with the maximum summation is assigned as the correct one as illustrated in the following equation:

$$Max\ S = \sum_{i=1} \mathrm{I}_{i\times Z} \tag{2}$$

where Z denotes a possible charge state and S is the

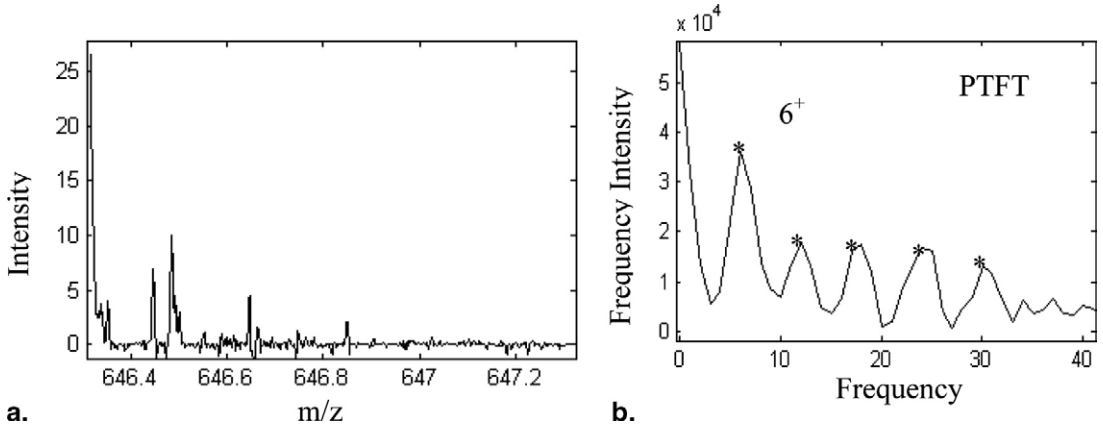

Figure 3. Peak-target Fourier transform of a noisy isotopic cluster in Figure 1c. (**a**) The "folded" spectrum after targeting the maximum peak at 646.315 *m/z*. (**b**) The frequency signal of the "folded" spectrum.

summation of intensities (I) of significant frequency peaks at the positions [Z, 2Z,···]. For example, the possible charge states contributing to the first peak in Figure 2d are 7^+, 8^+, and 9^+. Three summation values are obtained by eq 2. The maximum summation assigns 8^+ as the correct charge state.

Results and Discussion

High-performance mass spectrometers nowadays can generate a tremendous amount of high-resolution mass spectra. Charge state determination is very important for identification of isotopic clusters during the interpretation of a complex high-resolution mass spectrum. In particular, the rapid assignments of accurate charge states for complex overlapping isotopic clusters provide a great benefit in speed and accuracy for the analysis of high-resolution mass spectra. The performance of the PTFT algorithm is evaluated by comparing charge states determined by PTFT, AID-MS, and THRASH. The results show the robust capability of PTFT in charge state determination for low S/N and overlapping isotopic clusters. PTFT is useful to the manual verification of hidden isotopic clusters that are ignored by AID-MS or THRASH.

Low S/N Isotopic Clusters

When signal-to-noise ratio (S/N) is low, it is difficult to distinguish real isotopic peaks from noise signals. Using the reciprocal of interpeak spacing or Fourier transform to determine charge states may lead to ambiguous results. Figure 1c shows the outcome of a noisy isotopic cluster with the charge state 6^+ obtained from the results of AID-MS and THRASH. The frequency signal by FT exhibits only one significant peak (Figure 1d) without a clear pattern. When more noise signals are shown in a cluster and the S/N is lower, its frequency signal will totally lose the pattern for charge state determination. In this example, PTFT is applied the same spectrum by targeting the maximum peak. The "folded" spectrum and its frequency signal are shown in Figure 3a and b. It is noted that the "folding" strategy can enhance the S/N of isotopic clusters in the noisy spectrum. A frequency signal with a clear pattern can be unambiguously retrieved. Using all significant frequency peaks, we can easily determine the charge state (6^+) of this noisy isotopic cluster, which is in agreement with the results by AID-MS and THRASH.

Overlapping Isotopic Clusters

Overlapping isotopic clusters are often observed in high-resolution mass spectra of large biomolecules. Compared to THRASH, AID-MS has shown a robust ability to identify overlapping isotopic clusters. AID-MS uses a peak selection method and a cluster subtraction method to identify overlapping isotopic clusters according to the order of descending peak intensity. However, without the prior peak selection of isotopic peaks, PTFT can identify the charge states and peaks of overlapping isotopic clusters.

Figure 4a has three overlapping isotopic clusters identified by AID-MS. Each of the clusters corresponds to a specific charge state: 14^+, 9^+, and 7^+, respectively. As discussed previously [7], THRASH can identify only two of them (9^+ and 7^+) because the isotopic cluster 14^+ is too close to the cluster 9^+. The subtraction of the cluster 9^+ causes the deletion of one significant isotopic peak (Peak 2) in the cluster 14+. That is the reason why the cluster 14^+ is missed by THRASH. AID-MS can identify this cluster because it applies a robust cluster subtraction method that uses dynamic subtracting peak width.

The following analysis illustrates how PTFT automatically identifies the charge states of overlapping isotopic clusters. First, all peak maxima are picked and saved in a peak list. The maximum peak at 873.017 *m/z* (Peak 1) is selected as the first targeted peak. The PTFT result of this targeted peak is shown in Figure 4b. In the

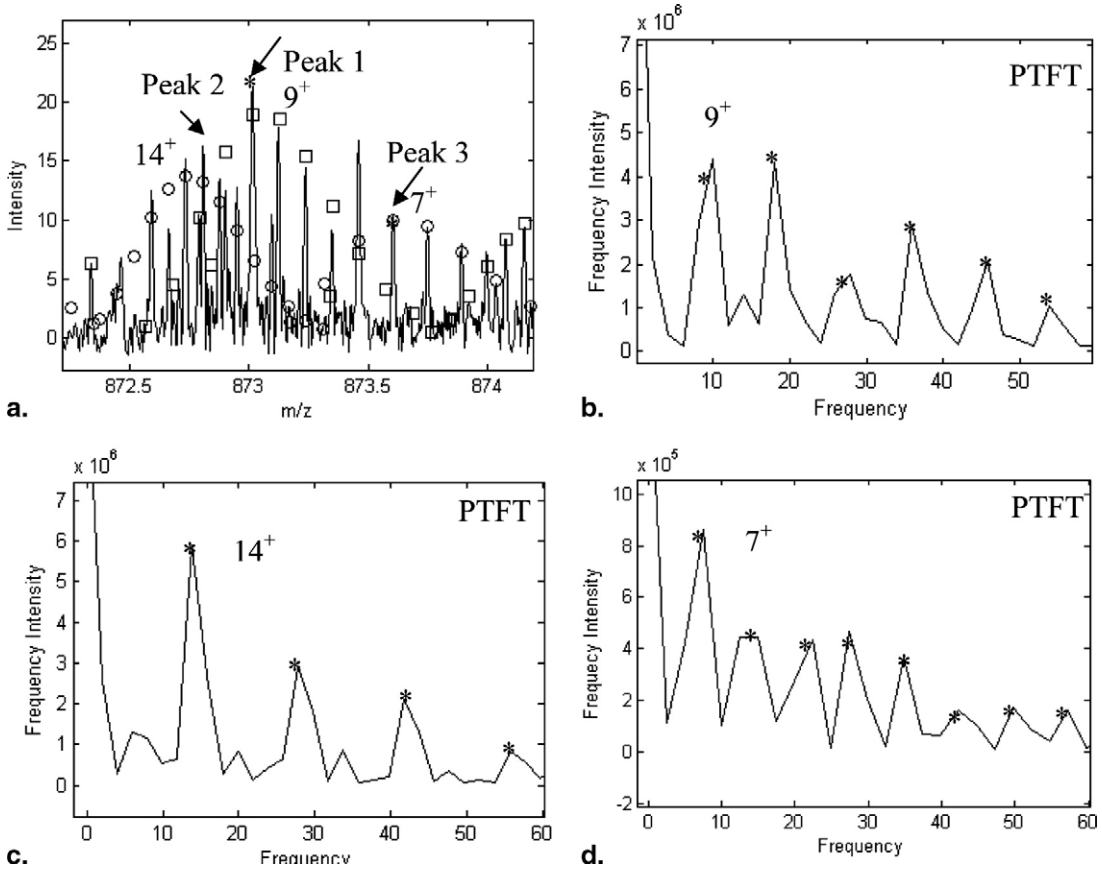

Figure 4. Peak-target Fourier transform of three overlapping isotopic clusters. (**a**) Three overlapping isotopic clusters identified by AID-MS; square and circle markers denote the neighboring matched *averagine* peaks. (**b**) The frequency signal of PTFT by targeting Peak 1. (**c**) The frequency signal of PTFT by targeting Peak 2. (**d**) The frequency signal of PTFT by targeting Peak 3.

frequency signal, the first peak is contributed by the charge states ranging from 6^+ to 11^+, and the first peak maximum is 10. This example shows that charge state assignment may be incorrect if using only the first peak maximum. All peak intensities with the interval of each possible charge state are summated. The maximum summation leads to the charge state 9^+ that is in consensus with the identification by AID-MS. All of isotopic peaks of this cluster are selected using Peak 1 and the reciprocal of the charge state (1/Z).

After removing the identified isotopic peaks from the peak list, the maximum peak at 872.808 *m/z* (Peak 2) in the remaining peak maxima is selected as the second targeted peak. PTFT is applied to the spectrum and the frequency signal is shown in Figure 4c. It is noteworthy that the subtraction of the cluster 9^+ from the spectrum is not required for the second PTFT. The charge state (14^+) and isotopic peaks of the second isotopic cluster can be easily identified. Similarly, the third isotopic cluster 7^+ is determined by the third targeted peak at 873.605 *m/z* (Peak 3). The frequency signal is shown in Figure 4d. In summary, three overlapping isotopic clusters are sequentially assigned by PTFT according to the order of descending peak intensity.

Manual Verification of Potential Hidden Isotopic Clusters

Although THRASH or AID-MS works well for the interpretation of high-resolution mass spectra, some isotopic clusters may remain hidden due to such phenomena as noise signals, overlapping complexity, or inappropriate cluster subtraction. For example, the weak clusters are often buried under their neighboring strong clusters in overlapping regions. To find all overlapping isotopic clusters is still a challenging task in the current analysis. Therefore, manual validation may be required to verify possible hidden isotopic clusters. A case in point is when users check through the identification results of a high-resolution mass spectrum obtained by the current analysis algorithms, they may still be suspicious of some pseudo-peaks that may be due to real isotopic clusters. For such suspected peaks, the users may need to manually select suspected isotopic peaks and then calculate their spacing to estimate their charge state. This procedure is troublesome and very time consuming. PTFT is very helpful for manual verification of suspicious isotopic peaks. It is very fast and easy to implement. The only requirement

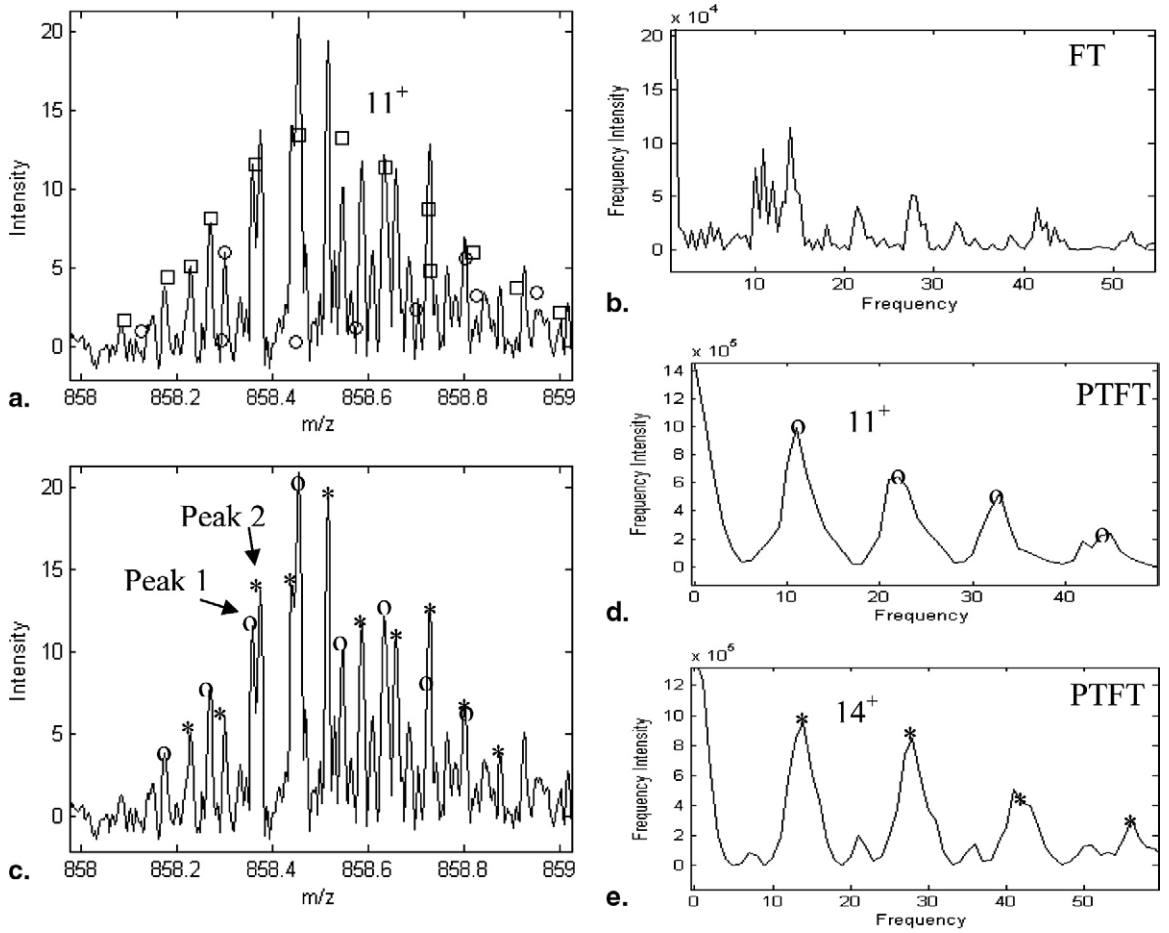

Figure 5. The detection of a hidden isotopic cluster by PTFT. (**a**) An *m/z* window with one isotopic cluster identified by AID-MS. Square markers denote the matched *averagine* peaks. (**b**) The frequency signal of FT. (**c**) Two identified isotopic clusters by PTFT. (**d**) The frequency signal of PTFT by targeting Peak 1. (**e**) The frequency signal of PTFT by targeting Peak 2.

for PTFT is to load the spectral data, select the targeted peak, and the *m/z* range they investigated.

Figure 5 illustrates an example for detection of a hidden isotopic cluster. As shown in Figure 5a, only one isotopic cluster with the charge state 11^+ is identified in the *m/z* range [858.0 859.0] by both AID-MS and THRASH. However, it is observed that some significant peaks are still not assigned. More than one cluster could be present in this overlapping region. The split peaks (Peak 1: 858.358 *m/z*; Peak 2: 858.372 *m/z*) are used as the targeted peaks for PTFT. As shown in Figure 5d, the frequency signal is found with a clear pattern (11^+) by targeting Peak 1. Isotopic peaks of this cluster are marked with symbol circle ("○") in Figure 5c. It is noted that this cluster is in agreement with the identified isotopic cluster 11^+ by AID-MS and THRASH. Figure 5e shows the frequency signal when targeting Peak 2. It has a clear pattern with 14^+, which indicates the existence of a new isotopic cluster. Isotopic peaks of this new cluster are identified and marked with symbol star ("*"). The missing of this cluster by AID-MS and THRASH is due to the inappropriate subtraction of the cluster 11^+, which has two highly overlapping isotopic peaks (858.358 *m/z* versus 858.372 *m/z* and 858.441 *m/z* versus 858.453 *m/z*) with the cluster 14^+. In summary, a hidden overlapping isotopic cluster is successfully found by PTFT.

Noise Signals and Isotopic Clusters with Low Charge States

Figure 6a–d illustrates an example for applying PTFT on a noise signal. From the frequency signals by FT and PTFT, we can see that no pattern is found in either signal, thus no isotopic cluster is indicated. In addition, an isotopic cluster with a low charge state is investigated as shown in Figure 6e–h. This cluster has only three isotopic peaks and the charge state is 3^+. In this case, the frequency signal by FT can be used to determine the charge state, although some noise peaks occur in the frequency signal. If the center peak is targeted, the frequency signal by PTFT is clean with a pattern of 3^+. In general, for low charge states, the number of isotopic peaks in one cluster could be few. If it is fewer than 3, PTFT is not recommended because no peak is found in the "folded" spectrum except the targeted

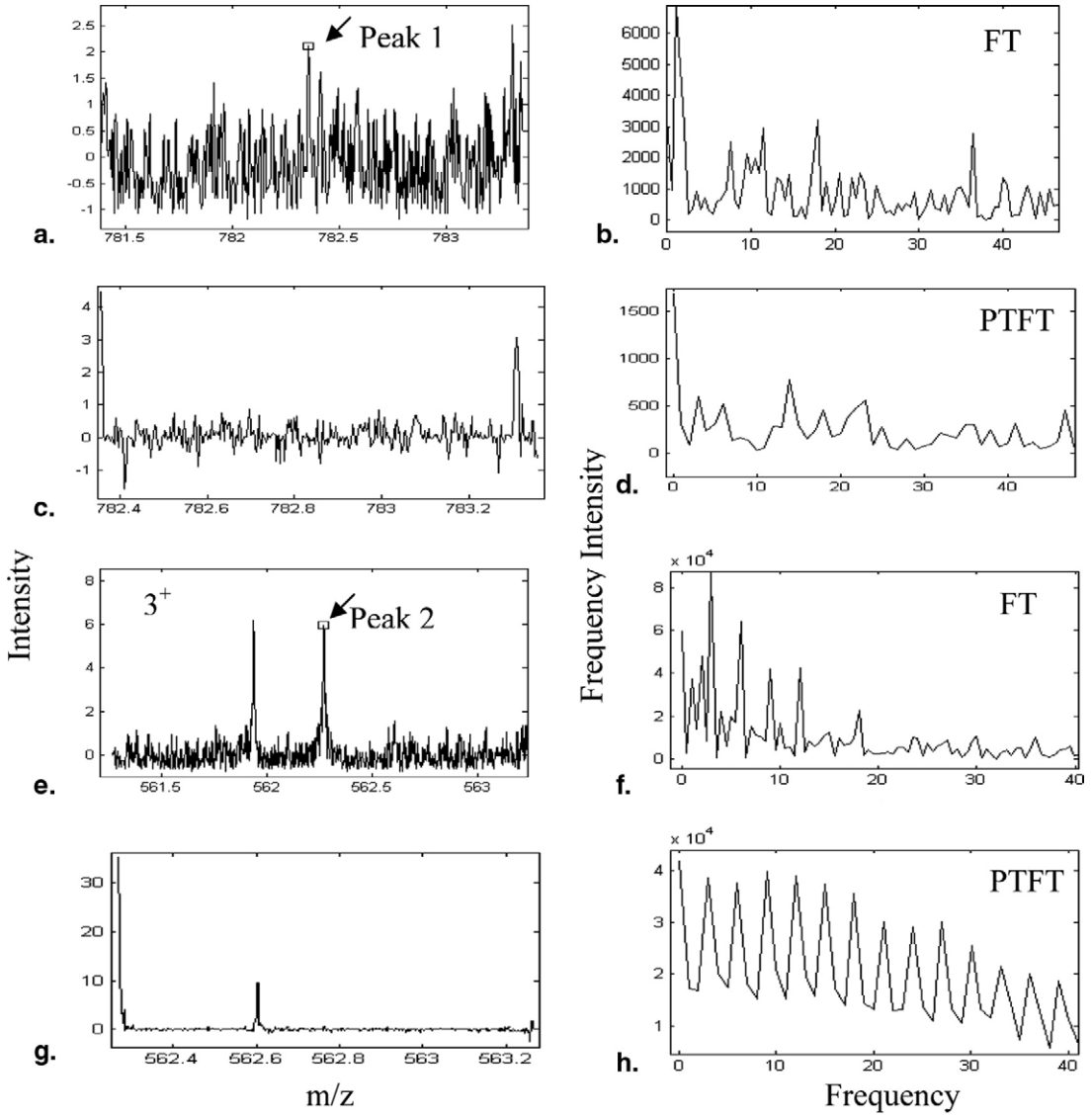

Figure 6. Peak-target Fourier transform of a noisy spectrum and an isotopic cluster with a low charge state. (1) A noisy spectrum (**a**) and its frequency signal of FT (**b**). (2) The "folded" spectrum by targeting Peak 1 (**c**) and its frequency signal (**d**). (3) An isotopic cluster with a low charge state 3^+ (**e**) and its frequency signal by FT (**f**). (4) The "folded" spectrum by targeting Peak 2 (**g**) and its frequency signal (**h**).

peak. However, for a very low charge state (1 or 2), the charge state can be easily determined by the reciprocal of the interpeak spacing as in AID-MS.

Conclusion

In this study, the feasibility—both theoretical and practical—of the PTFT algorithm has been demonstrated for charge state determination of complex isotope-resolved mass spectra. This algorithm applies a novel "folding" strategy to efficiently remove asymmetric peaks about a targeted peak and determines the charge state from the pattern in its frequency signal. The PTFT algorithm has shown robustness and speed for charge state determination of low S/N and overlapping isotopic clusters, and it is also useful for manual verification of potential hidden isotopic clusters. PTFT is not recommended for the case of a very low charge state. The fast determination of charge states for complex overlapping isotopic clusters by PTFT will provide a great benefit in speed and accuracy for the analysis of high-resolution mass spectra. As an extension, further research will aim at incorporating this algorithm to the current analysis algorithms for the interpretation of high-resolution mass spectra.

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